WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Tuesday, April 11, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L11	(thrABC near1 operon) same L6	0
☐	L10	(thrABC near1 operon) same L4	0
☐	L9	(thrABC near1 operon) same L1	17
☐	L8	threonine same L4	10
☐	L7	threonine same L2	14
☐	L6	L2 same L5	5
☐	L5	(microorganism or organism or enterobacteriaceae)same L1	1855
☐	L4	L1 same L2	25
☐	L2	((formate with C-acetyltransferase) or (pyruvate with formate-lyase)or (formate with acetyltransferase)or yfiD)	119
☐	L1	((L-amino near1 acid)or (amino near1 acid) same biosynthesis)	14405

END OF SEARCH HISTORY

=> index bioscience medicine

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 12:13:26 ON 11 APR 2006

73 FILES IN THE FILE LIST IN STNINDEX

```
=> s ((formate(w)C-acetyltransferase#) or (formate(w)acetyltransferase#)
or (pyruvate(w)formate-lyase) or yfiD)
     34  FILE AGRICOLA
      3  FILE AQUASCI
     51  FILE BIOENG
    295  FILE BIOSIS
     61  FILE BIOTECHABS
     61  FILE BIOTECHDS
    141  FILE BIOTECHNO
     42  FILE CABA
    377  FILE CAPLUS
     20  FILE CEABA-VTB
      2  FILE CIN
      5  FILE CONFSCI
      1  FILE DDFU
    189  FILE DGENE
 23 FILES SEARCHED...
     12  FILE DISSABS
      1  FILE DRUGU
      1  FILE EMBAL
    199  FILE EMBASE
    148  FILE ESBIOBASE
     11  FILE FEDRIP
      9  FILE FROSTI
     25  FILE FSTA
    510  FILE GENBANK
     28  FILE IFIPAT
      5  FILE JICST-EPLUS
    166  FILE LIFESCI
    261  FILE MEDLINE
      3  FILE NTIS
    109  FILE PASCAL
 51 FILES SEARCHED...
    408  FILE SCISEARCH
     64  FILE TOXCENTER
    151  FILE USPATFULL
     15  FILE USPAT2
     22  FILE WPIDS
      1  FILE WPIFV
     22  FILE WPINDEX
 70 FILES SEARCHED...

 36 FILES HAVE ONE OR MORE ANSWERS,  73 FILES SEARCHED IN STNINDEX

L1  QUE ((FORMATE(W) C-ACETYLTRANSFERASE#) OR (FORMATE(W) ACETYLTRANSFERASE#)
    OR (PYRUVATE(W) FORMATE-LYASE) OR YFID)

=> d rank
F1     510  GENBANK
F2     408  SCISEARCH
F3     377  CAPLUS
F4     295  BIOSIS
F5     261  MEDLINE
F6     199  EMBASE
F7     189  DGENE
F8     166  LIFESCI
F9     151  USPATFULL
F10    148  ESBIOBASE
F11    141  BIOTECHNO
```

```
F12     109  PASCAL
F13      64  TOXCENTER
F14      61  BIOTECHABS
F15      61  BIOTECHDS
F16      51  BIOENG
F17      42  CABA
F18      34  AGRICOLA
F19      28  IFIPAT
F20      25  FSTA

=> file f2-f6, f8-f13, f15-f16, f18, f21

FILE 'SCISEARCH' ENTERED AT 12:17:21 ON 11 APR 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'CAPLUS' ENTERED AT 12:17:21 ON 11 APR 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 12:17:21 ON 11 APR 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'MEDLINE' ENTERED AT 12:17:21 ON 11 APR 2006

FILE 'EMBASE' ENTERED AT 12:17:21 ON 11 APR 2006
Copyright (c) 2006 Elsevier B.V. All rights reserved.

FILE 'LIFESCI' ENTERED AT 12:17:21 ON 11 APR 2006
COPYRIGHT (C) 2006 Cambridge Scientific Abstracts (CSA)

FILE 'USPATFULL' ENTERED AT 12:17:21 ON 11 APR 2006
CA INDEXING COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'ESBIOBASE' ENTERED AT 12:17:21 ON 11 APR 2006
COPYRIGHT (C) 2006 Elsevier Science B.V., Amsterdam. All rights reserved.

FILE 'BIOTECHNO' ENTERED AT 12:17:21 ON 11 APR 2006
COPYRIGHT (C) 2006 Elsevier Science B.V., Amsterdam. All rights reserved.

FILE 'PASCAL' ENTERED AT 12:17:21 ON 11 APR 2006
Any reproduction or dissemination in part or in full,
by means of any process and on any support whatsoever
is prohibited without the prior written agreement of INIST-CNRS.
COPYRIGHT (C) 2006 INIST-CNRS. All rights reserved.

FILE 'TOXCENTER' ENTERED AT 12:17:21 ON 11 APR 2006
COPYRIGHT (C) 2006 ACS

FILE 'BIOTECHDS' ENTERED AT 12:17:21 ON 11 APR 2006
COPYRIGHT (C) 2006 THE THOMSON CORPORATION

FILE 'BIOENG' ENTERED AT 12:17:21 ON 11 APR 2006
COPYRIGHT (C) 2006 Cambridge Scientific Abstracts (CSA)

FILE 'AGRICOLA' ENTERED AT 12:17:21 ON 11 APR 2006

FILE 'WPIDS' ENTERED AT 12:17:21 ON 11 APR 2006
COPYRIGHT (C) 2006 THE THOMSON CORPORATION

=> s L1
L2      2487 L1

=> s (gene or sequence or polynucleotide or clone)(s)L2
  9 FILES SEARCHED...
L3       624 (GENE OR SEQUENCE OR POLYNUCLEOTIDE OR CLONE)(S) L2

=> s express?(s)L3
 10 FILES SEARCHED...
L4       250 EXPRESS?(S) L3
```

=> s ((L-amino (w) acid) or (amino (w) acid)(s) biosynthesis)(s)L4
2 FILES SEARCHED...
8 FILES SEARCHED...
11 FILES SEARCHED...
L7 9 ((L-AMINO (W) ACID) OR (AMINO (W) ACID)(S) BIOSYNTHESIS)(S) L4

=> s (microorganism or organism or enterobacteriaceae)(s)L4
L9 12 (MICROORGANISM OR ORGANISM OR ENTEROBACTERIACEAE)(S) L4

=> s (microorganism or organism or enterobacteriaceae)(s)L7
L10 4 (MICROORGANISM OR ORGANISM OR ENTEROBACTERIACEAE)(S) L7

=> s (thrABC(w)operon)(s)L4
L11 1 (THRABC(W) OPERON)(S) L4

=> dup rem L7
PROCESSING COMPLETED FOR L7
L12 5 DUP REM L7 (4 DUPLICATES REMOVED)

=> dup rem L9
PROCESSING COMPLETED FOR L9
L13 10 DUP REM L9 (2 DUPLICATES REMOVED)

=> dup rem L10
PROCESSING COMPLETED FOR L10
L14 3 DUP REM L10 (1 DUPLICATE REMOVED)

=> d ibib abs L12 1-5

L12 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-15593 BIOTECHDS
TITLE: Producing D-lactic acid, involves culturing bacteria having inactivated or lowered pyruvate formate-lyase activity in culture medium containing two or more amino acids and recovering lactic acid from culture;
lactic acid production via bacterium culture medium
AUTHOR: WADA M; OIKAWA T; MOCHIZUKI D; TOKUDA J; KAWASHIMA M; ARAKI T; ABE R; MIYAKE H; TAKAHASHI H; SAWAI H; MIMIZUKA T; MORISHIGE T; HIGASHI Y
PATENT ASSIGNEE: MITSUI CHEM INC
PATENT INFO: WO 2005033324 14 Apr 2005
APPLICATION INFO: WO 2004-JP14037 17 Sep 2004
PRIORITY INFO: JP 2004-150253 20 May 2004; JP 2003-340062 30 Sep 2003
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2005-315387 [32]
AN 2005-15593 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) lactic acid, involves culturing a heterolactic fermentation bacteria having inactivated or lowered ***pyruvate*** ***formate*** - ***lyase*** (pfl) activity in a culture medium containing two or more amino acids and recovering lactic acid from the culture.

DETAILED DESCRIPTION - Producing (M1): (a) lactic acid, involves culturing a heterolactic fermentation bacteria having inactivated or lowered ***pyruvate*** ***formate*** - ***lyase*** (pfl) activity in a culture medium containing two or more amino acids and recovering lactic acid from the culture; or (b) D-lactic acid, involves: (i) culturing a bacteria having inactivated or lowered pfl activity and elevated Escherichia coli-origin NADH-dependent D-lactic acid dehydrogenase (ldhA) activity and recovering D-lactic acid from the culture; or (ii) culturing microorganism having inactivated or lowered FAD-dependent D-lactic acid dehydrogenase (dld) activity in a liquid medium, producing and accumulating D-lactic acid in the culture solution and isolating D-lactic acid from the culture solution. INDEPENDENT CLAIMS are also included for: (1) microorganisms (I) having inactivated or lowered dld activity, pfl activity and/or enhanced ldhA activity; (2) microorganisms (II) having enhanced ldhA activity, where the activity is enhanced by ligating a ***gene*** encoding ldhA to a promoter of a

gene controlling the ***expression*** of a protein, which participate in a glycolysis system, a nucleic acid ***biosynthesis*** system or an ***amino*** ***acid*** ***biosynthesis*** system, on genome; and (3) microorganisms (III) having inactivated or lowered pfl and dld activity, and having a tricarboxylic acid (TCA) cycle and inactivated or lowered malate dehydrogenase (mdh) activity.

BIOTECHNOLOGY - Preferred Method: In (M1)-(a), the bacteria is E.coli MT-10934 (FERM BP-10057) strain. In (M1)-(b)-(i), the bacteria is E.coli and the culture medium contains two or more amino acids. In (M1), the culture is carried out on the following conditions, aerobic condition, 30degreesC temperature, pH ranging from 6-8 and oxygen volumetric coefficient (KLa) of 1 h-1 or more and 400 h-1 or less at normal pressure. Preferred Microorganism: (II) has inactivated or lowered dld and pfl activity. (II) is E.coli and the promoter is the promoter of glyceraldehyde 3 phosphate dehydrogenase ***gene*** derived from E.coli. (III) has inactivated or lowered aspartic acid ammonia-lyase (aspA) activity. (III) is E.coli and has enhanced ldhA activity.

USE - (M1) or (I)-(III) is useful for producing D-lactic acid. (III) is useful for producing compound (preferably D-lactic acid) other than organic acid produced in the TCA cycle, which involves culturing (III) in a culture medium and recovering the product (claimed).

ADVANTAGE - (M1) is a highly selected process that enables efficient production of D-lactic acid having a high optical purity with the formation of little organic acids as by products. (75 pages)

L12 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 1 ✓
ACCESSION NUMBER: 2004-25480 BIOTECHDS
TITLE: Production of L-amino acids e.g. L-threonine by fermentation involves culturing recombinant family Enterobacteriaceae microorganisms producing L-amino acid and having overexpressed yfiD open reading frame and/or pfl B gene; and isolating;
amino acid production via plasmid expression in host cell culture
AUTHOR: RIEPING M; FARWICK M
PATENT ASSIGNEE: DEGUSSA AG
PATENT INFO: WO 2004090149 21 Oct 2004
APPLICATION INFO: WO 2004-EP3207 26 Mar 2004
PRIORITY INFO: DE 2003-1016109 9 Apr 2003; DE 2003-1016109 9 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-757988 [74]
AN 2004-25480 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Production of L-amino acids by fermentation of recombinant Enterobacteriaceae involves culturing microorganisms producing ***L*** - ***amino*** ***acid*** and having overexpressed ***yfiD*** open reading frame (ORF) and/or pfl B ***gene*** or nucleotide sequences coding for the ***gene*** products; and isolating ***amino*** ***acid*** .

DETAILED DESCRIPTION - Production of L-amino acids by fermentation of recombinant microorganisms of Enterobacteriaceae family involves: (1) culturing microorganisms producing ***L*** - ***amino*** ***acid*** and having overexpressed ***yfiD*** open reading frame (ORF) and/or pfl B ***gene*** or nucleotide sequences coding for the ***gene*** products; and (2) isolating ***amino*** ***acid*** in which optional constituents of the fermentation broth and/or entire or portions (0 - 100%) of the biomass, optionally remain. INDEPENDENT CLAIMS are included for the following: (1) method (M) for the production of L-threonine involving fermenting microorganisms of Enterobacteriaceae family having enhanced genes for the biosynthetic pathway of L-threonine selected from at least one of: (a) the thrABC operon coding for aspartate kinase, homoserine dehydrogenase, homoserine kinase or threonine synthase; the pyc ***gene*** coding for pyruvate carboxylase; (b) the pps ***gene*** for phosphoenolpyruvate synthase; (c) the ppc ***gene*** coding for phosphoenolpyruvate carboxylase; (d) the genes pntA ad pntB coding for transhydrogenases; (e) the ***gene*** rhtB imparting homoserine resistance; (f) the mqo ***gene*** coding for malate:quinone oxidoreductase; (g) the

gene rhtC imparting threonine resistance; (h) the thrE ***gene*** coding for the threonine export protein; (i) the gdhA ***gene*** coding for glutamate dehydrogenase; (j) the hns ***gene*** coding for the DNA bonding protein HLP-II; (k) the pgm ***gene*** phosphoglucomutase; (l) the fba ***gene*** coding for fructose biphosphate aldolase; (m) the ptsH ***gene*** coding for phosphohistidine protein hexose phosphotranaferase; (n) the ptsI ***gene*** coding for enzyme I of the phosphotransferase system; (o) the crr ***gene*** coding for the glucose-specific IIA component; (p) the ptsG ***gene*** coding for the glucose-specific IIBC component; (q) the irp ***gene*** coding for the regulator of the leucine regulon; (r) the csrA ***gene*** coding for the global regulator Csr; (s) the fadA ***gene*** coding for the regulator of the fad regulon; (t) the iclR ***gene*** coding for the regulator of central intermediary metabolism; (u) the mopB ***gene*** coding for the 10 kDa chaperon; (v) the ahpC ***gene*** coding for the small subunit of alkyl hydroperoxide reductase; (w) the ahpF ***gene*** coding for the large subunit of alkyl hydroperoxide reductase; (x) the cysK ***gene*** coding for cysteine synthase A; (y) the cysB ***gene*** coding for the regulator of the cys regulon; (z) the cysJ ***gene*** coding for the flavoprotein of NADPH sulfite reductase; (aa) the cysH ***gene*** coding for adenylyl sulfate reductase; (bb) the phoR ***gene*** coding for the positive regulator PhoB of the pho regulon; (cc) the phoR ***gene*** coding for the sensor protein of the pho regulon; (dd) the phoE ***gene*** coding for the protein E of the outer cell membrane; (ee) the pykF ***gene*** coding for pyruvate kinase I, which is stimulated by fructose; (ff) the pfkB ***gene*** coding for 6-phosphofructokinase II; (gg) the male ***gene*** coding for the periplasmic binding protein of maltose transport; (hh) the soda ***gene*** coding for superoxide dismutase; (ii) the rseA ***gene*** coding for a membrane protein with anti-sigmaE activity; (jj) the rseC ***gene*** coding for a global regulator of the sigmaE factor; (kk) the sucA ***gene*** coding for the decarboxylase subunit of 2-ketoglutarate dehydrogenase; (ll) the sucB ***gene*** coding for the dihydrolipoyl transsuccinase E2 subunit of 2-ketoglutarate dehydrogenase; (mm) the sucC ***gene*** coding for the beta-subunit of succinyl-CoA synthetase; (nn) the sucD ***gene*** coding for the alpha-subunit of succinyl-CoA synthetase; (oo) the adk ***gene*** coding for adenylate kinase; (pp) the hdeA ***gene*** coding for a periplasmic protein with chaperonin-type function; (qq) the icd ***gene*** coding for isocitrate dehydrogenase; (rr) the mglB ***gene*** coding for the periplasmic galactose-binding transport protein; (ss) the lpd ***gene*** coding for dihydrolipoamide dehydrogenase; (tt) the aceE ***gene*** coding for the E1 component of the pyruvate-dehydrogenase complex; (uu) the sceF ***gene*** coding for the E2 component of the pyruvate-dehydrogenase complex; (vv) the pepB ***gene*** coding for aminopeptidase B; (ww) the aldH ***gene*** coding for aldehyde dehydrogenase; (xx) the bfr ***gene*** coding for the iron-storage homoprotein; (yy) the udp ***gene*** coding for uridine phosphorylase; or (zz) the resB ***gene*** coding for the regulator of sigmaE-factor activity; and (2) microorganisms of the Enterobacteriaceae family (preferably genus Escherichia)) in which the ***yfiD*** ORF and/or the pflB ***gene*** or nucleotide ***sequence*** coding for their ***gene*** product are present in enhanced or overexpressed form.

BIOTECHNOLOGY - Preferred Microorganisms: The recombinant microorganisms are generated by the transformation of a microorganism of the Enterobacteriaceae family with a vector containing ***yfiD*** ORF and/or p1fB ***gene*** ; so that the number of copies of the p1fB ***gene*** (s) and/or ***yfiD*** ORF is/are increased by at least 1. The ***yfiD*** ORF and/or pflB ***gene*** used is under the control of a promoter. Through the enhancement of the ***yfiD*** ORF and/or pflB ***gene*** , the concentration of activity of the ***yfiD*** ***gene*** product and/or the pfiB ***gene*** product (protein) is increased by at least 10%, relative to the activity or concentration of the ***gene*** product in the initial strain. The microorganism is selected from Escherichia, Erwinia, Providencia or Serratia. The microorganisms further have overexpressed genes of the biosynthetic pathway of the desired ***L*** - ***amino*** ***acid*** , and have the metabolic pathways that diminish the formation

of the desired ***L*** - ***amino*** ***acid*** , at least partially eliminated. In method (M). The microorganisms further have, simultaneously, ***gene*** selected from at least one of the ***gene*** coding for threonine dehydrogenase; the mdh ***gene*** coding for malate dehydrogenase, the ***gene*** product of the yifA ORF; the ***gene*** product of ytfP ORF; the pckA ***gene*** coding for phosphoenolpyruvate carboxykinase; the poxB ***gene*** coding for pyruvate oxidase; the aceA ***gene*** coding for isocitrate lyase; the dgsA ***gene*** coding for the DgsA regulator of the phosphotransferase system; the fruR ***gene*** coding for fructose repressor; the rpoS ***gene*** coding for the sigma38 factor; the aspA ***gene*** coding for aspartate ammonium lyase; or the aceB ***gene*** coding for malate synthase A, is/are attenuated, eliminated, or their ***expression*** is diminished. Preferred Method: The increase in the number of copies of the ***yfiD*** ORF and/or the pflB ***gene*** by at least 1 is achieved by extrachromosomal replication of the vector and involves: mutation of the promoter and regulation regions or the ribosome binding site upstream of the ***yfiD*** ORF and/or pflB ***gene*** ; or incorporating ***expression*** cassettes or promoters upstream of the ***yfiD*** ORF and/or pflB ***gene*** .

USE - For the production of L-amino acids (such as L-asparagine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine) by fermentation of recombinant microorganisms (claimed).

ADVANTAGE - The method results in an enhanced production of L-amino acids. The enhancement of ***yfiD*** ORF and pflB ***gene*** enhances the enzyme(s) of the known threonine- ***biosynthesis*** pathway, enzymes of the anaplerotic metabolism, enzymes for the production of reduced nicotinamide adenine dinucleotide phosphate, enzymes of glycolysis, PTS enzymes and/or enzymes of sulfur metabolism, and hence the production of L-amino acids.

EXAMPLE - Production of L-threonine with the strain MG422/pTrc99AyfiD was determined. The L-threonine producing E. coli strain MG442 as described in US278 was selected. The strain was transformed with an ***expression*** plasmid pTrc99AyfiD and with a vector pTrc99A and the plasmid bearing cells were selected on LB agar with ampicillin (50 microg/ml), to obtain strains MG443/pTrc99Ayfid and MG442/pTrc99A. The selected single colonies were subsequently multiplied further on minimal medium. The formation of threonine was examined in batch cultures (10 ml). At the end, a preculture medium (10 ml) was inoculated and incubated for 16 hours at 37 degrees C at shaker. At the time of preculture, the medium (250 micro l) was incubated into a production medium (10 ml) and incubated for 48 hours at 37 degrees C and the L-threonine formed was measured. Comparatively, the formation of L-threonine by initial strain MG442 was examined without the addition ampicillin. The L-threonine produced (g/l) by the strains MG442/pTrc99Ayfid/MG/pTrc99A/MG442 was found to be 2.5/1.3/1.4. (52 pages)

L12 ANSWER 3 OF 5 USPATFULL on STN
ACCESSION NUMBER: 2004:299255 USPATFULL
TITLE: Process for the production of L-amino acids using strains of the enterobacteriaceae family ✓
INVENTOR(S): Rieping, Mechthild, Bielefeld, GERMANY, FEDERAL REPUBLIC OF
Farwick, Mike, Essen, GERMANY, FEDERAL REPUBLIC OF

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004235122	A1	20041125
APPLICATION INFO.:	US 2004-817431	A1	20040405 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	DE 2003-10316109	20030409

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FITCH, EVEN, TABIN & FLANNERY, SUITE 401L, 1801 K

STREET, NW, WASHINGTON, DC, 20006-1201
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 1402
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a process for the production of L-amino acids by fermentation of recombinant microorganisms of the Enterobacteriaceae family, wherein

a) the yfiD ORF and/or the pflB gene or nucleotide sequences coding for the gene products are overexpressed in the microorganisms producing the desired L-amino acid, and the microorganisms are cultured in a medium under conditions in which the desired L-amino acid is enriched in the medium or in the cells; and

b) the desired L-amino acid is isolated, in a manner such that constituents of the fermentation broth and/or the biomass in its entirety or in portions (>0 to 100%) either remain in the isolated product or are completely removed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 4 OF 5 LIFESCI COPYRIGHT 2006 CSA on STN DUPLICATE 2
ACCESSION NUMBER: 2003:96408 LIFESCI
TITLE: Global Analyses of Transcriptomes and Proteomes of a Parent Strain and an L- Threonine-Overproducing Mutant Strain
AUTHOR: Lee, J.-H.; Lee, D.-E.; Lee, B.-U.; Kim, H.-S.*
CORPORATE SOURCE: 82-42-869-2616. Fax: 82-42-869-2610. E-mail:; E-mail: hskim@mail.kaist.ac.kr
SOURCE: Journal of Bacteriology [J. Bacteriol.], (20030900) vol. 185, no. 18, pp. 5442-5451.
ISSN: 0021-9193.
DOCUMENT TYPE: Journal
FILE SEGMENT: G; J
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We compared the transcriptome, proteome, and nucleotide sequences between the parent strain Escherichia coli W3110 and the L-threonine-overproducing mutant E. coli TF5015. DNA macroarrays were used to measure mRNA levels for all of the genes of E. coli, and two-dimensional gel electrophoresis was used to compare protein levels. It was observed that only 54 of 4,290 genes (1.3%) exhibited differential ***expression*** profiles. Typically, genes such as aceA, aceB, icdA, gltA, glnA, leu operon, proA, thrA, thrC, and yigJ, which are involved in the glyoxylate shunt, the tricarboxylic acid cycle, and ***amino*** ***acid*** ***biosynthesis*** (L-glutamine, L- leucine, proline, and L-threonine), were significantly upregulated, whereas the genes dadAX, hdeA, hdeB, ompF, oppA, oppB, oppF, ***yfiD*** , and many ribosomal protein genes were downregulated in TF5015 compared to W3110. The differential ***expression*** such as upregulation of thr operon and ***expression*** of yigJ would result in an accumulation of L-threonine in TF5015. Furthermore, two significant mutations, thrA345 and ilvA97, which are essential for overproduction of L-threonine, were identified in TF5015 by the ***sequence*** analysis. In particular, ***expression*** of the mutated thrABC (pATF92) in W3110 resulted in a significant incremental effect on L-threonine production. Upregulation of aceBA and downregulation of b1795, hdeAB, oppA, and ***yfiD*** seem to be linked to a low accumulation of acetate in TF5015. Such comprehensive analyses provide information regarding the regulatory mechanism of L-threonine production and the physiological consequences in the mutant stain.

L12 ANSWER 5 OF 5 USPATFULL on STN
ACCESSION NUMBER: 2002:291076 USPATFULL
TITLE: Polynucleotides, materials incorporating them, and methods for using them
INVENTOR(S): Glenn, Matthew, Auckland, NEW ZEALAND
Lubbers, Mark W., Palmerston North, NEW ZEALAND
Dekker, James, Palmerston North, NEW ZEALAND

PATENT ASSIGNEE(S): Genesis Research & Development Corporation Ltd., NEW ZEALAND (non-U.S. corporation)
Via Lactia BioScience (NZ) Ltd., NEW ZEALAND (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6476209	B1	20021105
APPLICATION INFO.:	US 2000-724623		20001128 (9)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Fredman, Jeffrey
ASSISTANT EXAMINER: Chakrabarti, Arun
LEGAL REPRESENTATIVE: Speckman, Ann W., Steath, Janet
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 0 Drawing Figure(s); 0 Drawing Page(s)
LINE COUNT: 5861
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel polynucleotides isolated from Lactobacillus rhamnosus, as well as probes and primers, genetic constructs comprising the polynucleotides, biological materials, including plants, microorganisms and multicellular organisms incorporating the polynucleotides, polypeptides expressed by the polynucleotides, and methods for using the polynucleotides and polypeptides are disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d ibib abs L13 1-10

L13 ANSWER 1 OF 10 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-15593 BIOTECHDS
TITLE: Producing D-lactic acid, involves culturing bacteria having inactivated or lowered pyruvate formate-lyase activity in culture medium containing two or more amino acids and recovering lactic acid from culture;
lactic acid production via bacterium culture medium
AUTHOR: WADA M; OIKAWA T; MOCHIZUKI D; TOKUDA J; KAWASHIMA M; ARAKI T; ABE R; MIYAKE H; TAKAHASHI H; SAWAI H; MIMIZUKA T; MORISHIGE T; HIGASHI Y
PATENT ASSIGNEE: MITSUI CHEM INC
PATENT INFO: WO 2005033324 14 Apr 2005
APPLICATION INFO: WO 2004-JP14037 17 Sep 2004
PRIORITY INFO: JP 2004-150253 20 May 2004; JP 2003-340062 30 Sep 2003
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2005-315387 [32]
AN 2005-15593 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) lactic acid, involves culturing a heterolactic fermentation bacteria having inactivated or lowered ***pyruvate*** ***formate*** - ***lyase*** (pfl) activity in a culture medium containing two or more amino acids and recovering lactic acid from the culture.

DETAILED DESCRIPTION - Producing (M1): (a) lactic acid, involves culturing a heterolactic fermentation bacteria having inactivated or lowered ***pyruvate*** ***formate*** - ***lyase*** (pfl) activity in a culture medium containing two or more amino acids and recovering lactic acid from the culture; or (b) D-lactic acid, involves: (i) culturing a bacteria having inactivated or lowered pfl activity and elevated Escherichia coli-origin NADH-dependent D-lactic acid dehydrogenase (ldhA) activity and recovering D-lactic acid from the culture; or (ii) culturing ***microorganism*** having inactivated or lowered FAD-dependent D-lactic acid dehydrogenase (dld) activity in a liquid medium, producing and accumulating D-lactic acid in the culture solution and isolating D-lactic acid from the culture solution.
INDEPENDENT CLAIMS are also included for: (1) microorganisms (I) having inactivated or lowered dld activity, pfl activity and/or enhanced ldhA activity; (2) microorganisms (II) having enhanced ldhA activity, where

the activity is enhanced by ligating a ***gene*** encoding ldhA to a promoter of a ***gene*** controlling the ***expression*** of a protein, which participate in a glycolysis system, a nucleic acid biosynthesis system or an amino acid biosynthesis system, on genome; and (3) microorganisms (III) having inactivated or lowered pfl and dld activity, and having a tricarboxylic acid (TCA) cycle and inactivated or lowered malate dehydrogenase (mdh) activity.

BIOTECHNOLOGY - Preferred Method: In (M1)-(a), the bacteria is E.coli MT-10934 (FERM BP-10057) strain. In (M1)-(b)-(i), the bacteria is E.coli and the culture medium contains two or more amino acids. In (M1), the culture is carried out on the following conditions, aerobic condition, 30degreesC temperature, pH ranging from 6-8 and oxygen volumetric coefficient (KLa) of 1 h-1 or more and 400 h-1 or less at normal pressure. Preferred ***Microorganism*** : (II) has inactivated or lowered dld and pfl activity. (II) is E.coli and the promoter is the promoter of glyceraldehyde 3 phosphate dehydrogenase ***gene*** derived from E.coli. (III) has inactivated or lowered aspartic acid ammonia-lyase (aspA) activity. (III) is E.coli and has enhanced ldhA activity.

USE - (M1) or (I)-(III) is useful for producing D-lactic acid. (III) is useful for producing compound (preferably D-lactic acid) other than organic acid produced in the TCA cycle, which involves culturing (III) in a culture medium and recovering the product (claimed).

ADVANTAGE - (M1) is a highly selected process that enables efficient production of D-lactic acid having a high optical purity with the formation of little organic acids as by products. (75 pages)

L13 ANSWER 2 OF 10 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 1

ACCESSION NUMBER: 2004-25480 BIOTECHDS

TITLE: Production of L-amino acids e.g. L-threonine by fermentation involves culturing recombinant family Enterobacteriaceae microorganisms producing L-amino acid and having overexpressed yfiD open reading frame and/or pf1B gene; and isolating;
amino acid production via plasmid expression in host cell culture

AUTHOR: RIEPING M; FARWICK M

PATENT ASSIGNEE: DEGUSSA AG

PATENT INFO: WO 2004090149 21 Oct 2004

APPLICATION INFO: WO 2004-EP3207 26 Mar 2004

PRIORITY INFO: DE 2003-1016109 9 Apr 2003; DE 2003-1016109 9 Apr 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-757988 [74]

AN 2004-25480 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Production of L-amino acids by fermentation of recombinant ***Enterobacteriaceae*** involves culturing microorganisms producing L-amino acid and having overexpressed ***yfiD*** open reading frame (ORF) and/or pf1B ***gene*** or nucleotide sequences coding for the ***gene*** products; and isolating amino acid.

DETAILED DESCRIPTION - Production of L-amino acids by fermentation of recombinant microorganisms of ***Enterobacteriaceae*** family involves: (1) culturing microorganisms producing L-amino acid and having overexpressed ***yfiD*** open reading frame (ORF) and/or pf1B ***gene*** or nucleotide sequences coding for the ***gene*** products; and (2) isolating amino acid in which optional constituents of the fermentation broth and/or entire or portions (0 - 100%) of the biomass, optionally remain. INDEPENDENT CLAIMS are included for the following: (1) method (M) for the production of L-threonine involving fermenting microorganisms of ***Enterobacteriaceae*** family having enhanced genes for the biosynthetic pathway of L-threonine selected from at least one of: (a) the thrABC operon coding for aspartate kinase, homoserine dehydrogenase, homoserine kinase or threonine synthase; the pyc ***gene*** coding for pyruvate carboxylase; (b) the pps ***gene*** for phosphoenolpyruvate synthase; (c) the ppc ***gene*** coding for phosphoenolpyruvate carboxylase; (d) the genes pntA ad pntB coding for transhydrogenases; (e) the ***gene*** rhtB imparting homoserine resistance; (f) the mqo ***gene*** coding for

malate:quinone oxidoreductase; (g) the ***gene*** rhtC imparting threonine resistance; (h) the thrE ***gene*** coding for the threonine export protein; (i) the gdhA ***gene*** coding for glutamate dehydrogenase; (j) the hns ***gene*** coding for the DNA bonding protein HLP-II; (k) the pgm ***gene*** phosphoglucomutase; (l) the fba ***gene*** coding for fructose biphosphate aldolase; (m) the ptsH ***gene*** coding for phosphohistidine protein hexose phosphotranaferase; (n) the ptsI ***gene*** coding for enzyme I of the phosphotransferase system; (o) the crr ***gene*** coding for the glucose-specific IIA component; (p) the ptsG ***gene*** coding for the glucose-specific IIBC component; (q) the irp ***gene*** coding for the regulator of the leucine regulon; (r) the csrA ***gene*** coding for the global regulator Csr; (s) the fadA ***gene*** coding for the regulator of the fad regulon; (t) the iclR ***gene*** coding for the regulator of central intermediary metabolism; (u) the mopB ***gene*** coding for the 10 kDa chaperon; (v) the ahpC ***gene*** coding for the small subunit of alkyl hydroperoxide reductase; (w) the ahpF ***gene*** coding for the large subunit of alkyl hydroperoxide reductase; (x) the cysK ***gene*** coding for cysteine synthase A; (y) the cysB ***gene*** coding for the regulator of the cys regulon; (z) the cysJ ***gene*** coding for the flavoprotein of NADPH sulfite reductase; (aa) the cysH ***gene*** coding for adenylyl sulfate reductase; (bb) the phoR ***gene*** coding for the positive regulator PhoB of the pho regulon; (cc) the phoR ***gene*** coding for the sensor protein of the pho regulon; (dd) the phoE ***gene*** coding for the protein E of the outer cell membrane; (ee) the pykF ***gene*** coding for pyruvate kinase I, which is stimulated by fructose; (ff) the pfkB ***gene*** coding for 6-phosphofructokinase II; (gg) the male ***gene*** coding for the periplasmic binding protein of maltose transport; (hh) the soda ***gene*** coding for superoxide dismutase; (ii) the rseA ***gene*** coding for a membrane protein with anti-sigmaE activity; (jj) the rseC ***gene*** coding for a global regulator of the sigmaE factor; (kk) the sucA ***gene*** coding for the decarboxylase subunit of 2-ketoglutarate dehydrogenase; (ll) the sucB ***gene*** coding for the dihydrolipoyl transsuccinase E2 subunit of 2-ketoglutarate dehydrogenase; (mm) the sucC ***gene*** coding for the beta-subunit of succinyl-CoA synthetase; (nn) the sucD ***gene*** coding for the alpha-subunit of succinyl-CoA synthetase; (oo) the adk ***gene*** coding for adenylate kinase; (pp) the hdeA ***gene*** coding for a periplasmic protein with chaperonin-type function; (qq) the icd ***gene*** coding for isocitrate dehydrogenase; (rr) the mglB ***gene*** coding for the periplasmic galactose-binding transport protein; (ss) the lpd ***gene*** coding for dihydrolipoamide dehydrogenase; (tt) the aceE ***gene*** coding for the E1 component of the pyruvate-dehydrogenase complex; (uu) the sceF ***gene*** coding for the E2 component of the pyruvate-dehydrogenase complex; (vv) the pepB ***gene*** coding for aminopeptidase B; (ww) the aldH ***gene*** coding for aldehyde dehydrogenase; (xx) the bfr ***gene*** coding for the iron-storage homoprotein; (yy) the udp ***gene*** coding for uridine phosphorylase; or (zz) the resB ***gene*** coding for the regulator of sigmaE-factor activity; and (2) microorganisms of the ***Enterobacteriaceae*** family (preferably genus Escherichia)) in which the ***yfiD*** ORF and/or the pflB ***gene*** or nucleotide ***sequence*** coding for their ***gene*** product are present in enhanced or overexpressed form.

BIOTECHNOLOGY - Preferred Microorganisms: The recombinant microorganisms are generated by the transformation of a ***microorganism*** of the ***Enterobacteriaceae*** family with a vector containing ***yfiD*** ORF and/or plfB ***gene*** ; so that the number of copies of the plfB ***gene*** (s) and/or ***yfiD*** ORF is/are increased by at least 1. The ***yfiD*** ORF and/or pflB ***gene*** used is under the control of a promoter. Through the enhancement of the ***yfiD*** ORF and/or pflB ***gene*** , the concentration of activity of the ***yfiD*** ***gene*** product and/or the pfiB ***gene*** product (protein) is increased by at least 10%, relative to the activity or concentration of the ***gene*** product in the initial strain. The ***microorganism*** is selected from Escherichia, Erwinia, Providencia or Serratia. The microorganisms further have overexpressed genes of the biosynthetic pathway of the desired L-amino acid, and have the metabolic pathways that diminish the

formation of the desired L-amino acid, at least partially eliminated. In method (M). The microorganisms further have, simultaneously, ***gene*** selected from at least one of the ***gene*** coding for threonine dehydrogenase; the mdh ***gene*** coding for malate dehydrogenase, the ***gene*** product of the yifA ORF; the ***gene*** product of ytfP ORF; the pckA ***gene*** coding for phosphoenolpyruvate carboxykinase; the poxB ***gene*** coding for pyruvate oxidase; the aceA ***gene*** coding for isocitrate lyase; the dgsA ***gene*** coding for the DgsA regulator of the phosphotransferase system; the fruR ***gene*** coding for fructose repressor; the rpoS ***gene*** coding for the sigma38 factor; the aspA ***gene*** coding for aspartate ammonium lyase; or the aceB ***gene*** coding for malate synthase A, is/are attenuated, eliminated, or their ***expression*** is diminished. Preferred Method: The increase in the number of copies of the ***yfiD*** ORF and/or the pflB ***gene*** by at least 1 is achieved by extrachromosomal replication of the vector and involves: mutation of the promoter and regulation regions or the ribosome binding site upstream of the ***yfiD*** ORF and/or pflB ***gene*** ; or incorporating ***expression*** cassettes or promoters upstream of the ***yfiD*** ORF and/or pflB ***gene*** .

USE - For the production of L-amino acids (such as L-asparagine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine) by fermentation of recombinant microorganisms (claimed).

ADVANTAGE - The method results in an enhanced production of L-amino acids. The enhancement of ***yfiD*** ORF and pflB ***gene*** enhances the enzyme(s) of the known threonine-biosynthesis pathway, enzymes of the anaplerotic metabolism, enzymes for the production of reduced nicotinamide adenine dinucleotide phosphate, enzymes of glycolysis, PTS enzymes and/or enzymes of sulfur metabolism, and hence the production of L-amino acids.

EXAMPLE - Production of L-threonine with the strain MG422/pTrc99AyfiD was determined. The L-threonine producing E. coli strain MG442 as described in US278 was selected. The strain was transformed with an ***expression*** plasmid pTrc99AyfiD and with a vector pTrc99A and the plasmid bearing cells were selected on LB agar with ampicillin (50 microg/ml), to obtain strains MG443/pTrc99Ayfid and MG442/pTrc99A. The selected single colonies were subsequently multiplied further on minimal medium. The formation of threonine was examined in batch cultures (10 ml). At the end, a preculture medium (10 ml) was inoculated and incubated for 16 hours at 37 degrees C at shaker. At the time of preculture, the medium (250 micro l) was incubated into a production medium (10 ml) and incubated for 48 hours at 37 degrees C and the L-threonine formed was measured. Comparatively, the formation of L-threonine by initial strain MG442 was examined without the addition ampicillin. The L-threonine produced (g/l) by the strains MG442/pTrc99Ayfid/MG/pTrc99A/MG442 was found to be 2.5/1.3/1.4. (52 pages)

L13 ANSWER 3 OF 10 USPATFULL on STN
ACCESSION NUMBER: 2004:299255 USPATFULL
TITLE: Process for the production of L-amino acids using strains of the enterobacteriaceae family
INVENTOR(S): Rieping, Mechthild, Bielefeld, GERMANY, FEDERAL REPUBLIC OF
Farwick, Mike, Essen, GERMANY, FEDERAL REPUBLIC OF

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004235122	A1	20041125
APPLICATION INFO.:	US 2004-817431	A1	20040405 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	DE 2003-10316109	20030409

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FITCH, EVEN, TABIN & FLANNERY, SUITE 401L, 1801 K STREET, NW, WASHINGTON, DC, 20006-1201

NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)
LINE COUNT: 1402
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a process for the production of L-amino acids by fermentation of recombinant microorganisms of the Enterobacteriaceae family, wherein

a) the yfiD ORF and/or the pflB gene or nucleotide sequences coding for the gene products are overexpressed in the microorganisms producing the desired L-amino acid, and the microorganisms are cultured in a medium under conditions in which the desired L-amino acid is enriched in the medium or in the cells; and

b) the desired L-amino acid is isolated, in a manner such that constituents of the fermentation broth and/or the biomass in its entirety or in portions (>0 to 100%) either remain in the isolated product or are completely removed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 4 OF 10 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2004-736360 [72] WPIDS
DOC. NO. NON-CPI: N2004-582720
DOC. NO. CPI: C2004-258817
TITLE: Use of enzymes involved in Acetyl Coenzyme A (A-CoA) metabolism for manipulating the metabolism of a cell, increasing the A-CoA flux in a cell, increasing the A-CoA pools in a cell, or producing isoamyl acetate in a cell.
DERWENT CLASS: B04 D16 P13
INVENTOR(S): BENNETT, G N; SAN, K; VADALI, R V
PATENT ASSIGNEE(S): (RICV) UNIV RICE
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND DATE	WEEK	LA	PG
US 2004199941	A1 20041007	(200472)*		19

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2004199941	A1 Provisional	US 2003-457093P	20030324
	Provisional	US 2003-457635P	20030326
		US 2004-808717	20040324

PRIORITY APPLN. INFO: US 2004-808717 20040324; US 2003-457093P 20030324; US 2003-457635P 20030326
AN 2004-736360 [72] WPIDS
AB US2004199941 A UPAB: 20041109
NOVELTY - Using enzymes involved in Acetyl Coenzyme A (A-CoA) metabolism for manipulating the metabolism of a cell, increasing the A-CoA flux in a cell, increasing the A-CoA pools in a cell, producing isoamyl acetate in a cell, increasing CoA pools, or increasing synthesis of CoA containing compounds from a bacterial cell, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:
(1) manipulating the metabolism of a cell;
(2) increasing the A-CoA flux in a cell;
(3) increasing the A-CoA pools in a cell;
(4) biosynthesis of one or more target compounds;
(5) producing isoamyl acetate in a cell;
(6) a microorganism which expresses one or more enzymes involved in A-CoA metabolism at elevated levels, where the microorganism displays increased flux through the A-CoA node;
(7) increasing CoA pools; and

(8) increasing synthesis of CoA containing compounds from a bacterial cell.

USE - The enzymes involved in A-CoA metabolism are useful for manipulating the metabolism of a cell, increasing the A-CoA flux in a cell, increasing the A-CoA pools in a cell, producing isoamyl acetate in a cell, increasing CoA pools, or increasing synthesis of CoA containing compounds from a bacterial cell.

Dwg.0/11

L13 ANSWER 5 OF 10 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN DUPLICATE 2

ACCESSION NUMBER: 2003-10747 BIOTECHDS

TITLE: Method for the production of pyruvate in high conversion and yield, comprises carrying out fermentation using microorganisms with reduced or eliminated activity of pyruvate decomposing enzymes;
pyruvic acid preparation by mutant bacterium fermentation

AUTHOR: BOTT M; GERHARZ T; TAKORS R; ZELIC B

PATENT ASSIGNEE: FORSCHUNGSZENTRUM JUELICH GMBH

PATENT INFO: WO 2003000913 3 Jan 2003

APPLICATION INFO: WO 2002-DE2144 12 Jun 2002

PRIORITY INFO: DE 2001-1029711 22 Jun 2001; DE 2001-1029711 22 Jun 2001

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 2003-239147 [23]

AN 2003-10747 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - New method for the production of pyruvate (I) comprises carrying out fermentation using microorganisms with reduced or completely eliminated activity of (I)-decomposing enzymes.

BIOTECHNOLOGY - Preferred Microorganisms: The microorganisms have the nucleotide ***sequence*** (s) encoding one or more of the following deleted, not ***expressed*** or ***expressed*** less strongly than in the natural ***sequence*** : pyruvate dehydrogenase, pyruvate decarboxylase, pyruvate : ferredoxin oxido-reductase, pyruvate : flavodoxin oxido-reductase, ***pyruvate*** - ***formate*** ***lyase*** , phosphoenol-pyruvate synthetase and/or pyruvate oxidase; in particular the microorganisms lack or are deficient in the aceE and aceF genes, the poxB ***gene*** , the pps ***gene*** and/or the pflB ***gene*** . The microorganisms are of the ***Enterobacteriaceae*** group, preferably of the Escherichia genus, especially Escherichia coli, particularly Escherichia YYC 202 (DSM 14335). Preferred Process: Fermentation is carried out anerobically or aerobically, with addition of carbohydrates, organic acids or alcohols as substrate. Preferably, acetate is added in the case of fermentation with growing cells and acetate is not added in the case of fermentation with quiescent cells. The fermentation is monitored by carbon dioxide analysis.

USE - For the production of pyruvate (I).

ADVANTAGE - (I) can be obtained at almost 100% substrate conversion and with maximized yield. The process is simpler and less expensive than chemical synthesis of (I).

EXAMPLE - Escherichia YYC 202 (DSM 14335) (a genetically modified strain lacking or deficient in the aceE ***gene*** (encoding the E1 and E2 sub-units of the pyruvate dehydrogenase complex), the poxB ***gene*** (encoding pyruvate oxidase), the pps ***gene*** (encoding phosphoenol-pyruvate synthetase) and the pflB ***gene*** (encoding ***pyruvate*** - ***formate*** ***lyase***) was cultured in M9 minimal medium in presence of glucose and acetate. The cells were recovered by centrifugation, washed twice with 1/10 culture volumes of 0.9% sodium chloride solution and resuspended in buffer (MES/MOPS/tricine) of pH 6.5 containing 10 mM glucose but no acetate or other nutrients. The quiescent cell suspension was incubated for 24 hours at 37 degrees C under rotational shaking at 140 rpm. Analysis of the supernatant showed that pyruvate (I) was obtained in a yield of 1.9 mole per mole of glucose (compared with a theoretical maximum of 2 moles per mole). (29 pages)

L13 ANSWER 6 OF 10 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2002-19581 BIOTECHDS

TITLE: Novel isolated Lactobacillus rhamnosus polynucleotides encoding polypeptide with anti-infection/lactose digestion modulating activity, useful to improve properties of microbes used in milk-derived products manufacture; vector-mediated recombinant protein gene transfer and expression in host cell for use in recombinant vaccine preparation and as a probiotic and food-additive

AUTHOR: GLENN M; HAVUKKALA I J; LUBBERS M W; DEKKER J

PATENT ASSIGNEE: GENESIS RES and DEV CORP LTD; VIALACTIA BIOSCIENCE NZ LTD

PATENT INFO: WO 2002044383 6 Jun 2002

APPLICATION INFO: WO 2000-NZ286 28 Nov 2000

PRIORITY INFO: US 2000-724623 28 Nov 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-519588 [55]

AN 2002-19581 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - An isolated ***polynucleotide*** (I) comprising a nucleotide ***sequence*** present in Lactobacillus rhamnosus strain HN001 that encodes a polypeptide with activity such as enzyme activity; anti-infection activity; lactose digestion modulating activity; immune system modulating activity; amino acid, lipid, vitamin or carbohydrate metabolic activity, flavor, texture or aroma modulating activity, is new.

DETAILED DESCRIPTION - An isolated ***polynucleotide*** (I) comprises a nucleotide ***sequence*** present in Lactobacillus rhamnosus strain HN001 that encodes a polypeptide with activity such as enzyme activity; anti-infection activity; lactose digestion modulating activity; immune system modulating activity; amino acid, lipid, vitamin or carbohydrate metabolic activity, flavor, texture or aroma modulating activity, is new. In addition, multistress resistance and survival activity; antigenic activity; adhesion activity; or regulatory activity. INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** (II) comprising a nucleotide ***sequence*** which is a: (i) complement of a fully defined ***sequence*** (NS) of any one of 59 (400-2100) base pair sequences all given in the specification; (ii) reverse complement of NS; or (iii) reverse sequences of NS; (2) an isolated ***polynucleotide*** (III) comprising a nucleotide ***sequence*** having 200-mer (at least 40-mer) of NS or its variant; (3) an isolated ***polynucleotide*** (IV) of (I) comprising a nucleotide ***sequence*** that differs from NS as a result of: (i) conservative substitutions; or (ii) deletions and/or insertions and/or substitutions totaling less than 10% or 15% of the total ***sequence*** length; (4) an isolated polypeptide (V) encoded by any one of the above mentioned isolated polynucleotides. (V) comprises: (i) an amino acid ***sequence*** (PS) of any one of 59 (150-700) amino acid sequences all given in the specifications; (ii) sequences producing an producing an Expectation (E) value of 0.01 or less when compared to PS using the BLASTP algorithm; (iii) sequences comprising an amino acid ***sequence*** having at least 75% identity to PS or its variant; (iv) sequences differing by codon alterations that reflect the degeneracy of the genetic code; and (v) functionally similar sequences differing only by conservative amino acid substitutions; (5) a fusion protein (VI) comprising (V); (6) a kit comprising several oligonucleotide probes or primers comprising at least 10 contiguous residues complementary to 10 contiguous residues of NS or its variant; (7) a genetic construct (VII) comprising NS or its variant; (8) a transgenic cell (VIII) comprising (VII); and (9) a genetic construct (IX) comprising, in the 5'-3' direction: (a) a ***gene*** promoter ***sequence*** ; (b) a ***polynucleotide*** ***sequence*** comprising a ***polynucleotide*** coding for at least a functional portion of a polypeptide encoded by NS or its variant, or a ***polynucleotide*** comprising a non-coding region of a ***gene*** coding for an polypeptide encoded by NS or its variant; and (c) a ***gene*** termination ***sequence*** .

WIDER DISCLOSURE - The following are disclosed: (1) transgenic organisms such as microbes comprising (VIII); (2) polynucleotides that differ from (I) due to degeneracy of genetic code; (3) oligonucleotide probes and primers complementary to and/or corresponding to NS; (4) collection of several (I) and (V); (5) storage medium comprising the above mentioned collection; (6) a combination of polynucleotides which

comprise at least 50 different polynucleotides and/or polypeptides and variants of the polynucleotides and polypeptides; and (7) transgenic plant cells comprising (VII) or (IX).

BIOTECHNOLOGY - Preferred ***Polynucleotide*** : (I) has a ***sequence*** of NS, sequences comprising a nucleotide ***sequence*** producing an Expectation (E) value of 0.01 or less when compared to NS using the BLASTN algorithm version 2.04 set to the default parameters described in the specification. (I) comprises a nucleotide ***sequence*** having at least 75% identical nucleotides to a reference ***sequence*** of any one of NS. Optionally (I) hybridizes to a ***polynucleotide*** comprising a ***sequence*** of NS under hybridization conditions. Preferred Genetic Construct: (VII) comprises a ***polynucleotide*** encoding a polypeptide that modifies the flavor, aroma, texture and health-related benefits of milk-derived products, where the polypeptide is histidinol-phosphate aminotransferase, tyrosine aminotransferase, cysteine desulfurase, lipase, O-acetylserine sulfhydrylase, surface protein, Group B streptococcal oligopeptidase, Pz-peptidase, dipeptidase, acylaminoacyl-peptidase, carboxylesterase, glycerophosphodiester phosphodiesterase, bifunctional alcohol dehydrogenase and acetaldehyde dehydrogenase, short-chain alcohol dehydrogenase, branched chain amino acid transport system II carrier protein, malolactic enzyme, pyruvate dehydrogenase, E1 (lipoamide) alpha subunit, ***formate*** ***C*** - ***acetyltransferase*** , 6-phosphogluconate dehydrogenase, 5-methyltetrahydropteroyltriglutamate homocysteine methyltransferase, S-methylmethionine permease, 6-phospho-beta-galactosidase, gamma-glutamyl phosphate reductase, dihydrofolate reductase, lactate dehydrogenase, lysine, aminopeptidase pepS, fibronectin binding protein AB2, prolinase (pepR), Xaa-Pro dipeptidase or human bile salt export pump. Preferably the ***polynucleotide*** encodes a polypeptide that increases the survivability of a microbe used in the manufacture of dairy products and probiotic supplements, where the polypeptide is any one of transmembrane adhesion protein, major cell adherence molecule of Campylobacter jejuni and Campylobacter coli, collagen/mucin binding protein, ATP-dependent ClpC proteinase regulatory protein, O-sialoglycoprotein endopeptidase, human bile salt export pump, adhesion, bifunctional HPr Kinase/P- Ser-HPr phosphatase, malolactic enzyme, magnesium transporter, dTDP-4-keto-L-rhamnose reductase, glucose inhibited division protein, glucose-1-phosphate thymidylyl transferase, phosphate starvation-induced protein, GTP binding protein, gamma-glutamyl phosphate reductase, Pav A, aminopeptidase pepS, heat-inducible transcription repressor protein, phosphoribosylaminioimidazolecarboxyamide formyltransferase/imp cyclohydrolase, hexulose-6-phosphate isomerase and fibronectin binding protein AB2. Preferred Genetic Construct: (IX) comprises ***gene*** promoter and ***gene*** termination sequences which are functional in a prokaryote or eukaryote.

ACTIVITY - Vulnerary; Antilipaemic; Immunostimulant. No supporting data is given.

MECHANISM OF ACTION - Vaccine.

USE - (I) is useful for improving the properties of microbes used in the manufacture of milk-derived products and probiotic supplements, which involves modulating the ***polynucleotide*** content or composition of the microbes by transforming the microbes with (I). (I) having a ***sequence*** of NS is also useful for identifying an ***organism*** (preferably a bacterial or yeast cell) or reproductive material or an extract from the ***organism*** , as having a specific origin, which involves detecting in the genetic complement of the ***organism*** , material or extract the presence or absence of (I) that is representative of the origin, and has a ***sequence*** of NS. The presence or absence of (I) is identified by isolating DNA from the ***organism*** or material and contacting the isolated DNA with at least one oligonucleotide probe specific for (I). Optionally the isolated DNA is contacted with several oligonucleotide probes in an array format. (V) encoded by (I) is also useful for modifying the flavor, aroma, texture and/or nutritional and health benefits of milk-derived products, which involves adding one or more polypeptides to the milk being processed. (VII) or (IX) is useful for modulating the ***polynucleotide*** content or composition of a ***organism*** (all claimed). (I) is useful for identifying, isolating or synthesizing DNA molecules such as promoter, DNA binding elements, open reading frames or full-length genes,

that then can be used as ***expressible*** DNA in transgenic organisms. (I) may be used to detect lactic acid bacteria, preferably L.rhamnosus in a sample material. (I) is also useful for genome mapping, physical mapping, and in positional cloning of genes of more or less related microbes, and to design probes and primers. (I) is also useful for transforming microbes for use in a therapeutic composition that is effective for treating or preventing a gastrointestinal condition or disorder caused by the presence of pathogenic microbes in the gastrointestinal tract or by the absence of normal intestinal microbes in the intestinal tract. (V) is used to raise antibodies, to isolate corresponding interacting proteins, as nutritional additives and as additives in dairy processing and fermentation processing. (I) and (V) are used for the selection and production of more effective probiotic bacteria, as bioactive (health promoting) ingredients and health supplements, for immune function enhancement; for reduction of blood lipids such as cholesterol; for production of bioactive material from genetically modified bacteria; as adjuvants; for wound healing; in vaccine development, in selection and production of genetically modified rumen microorganisms for improved animal nutrition and productivity, better flavor and improved milk composition. (VII) is useful for modifying concentration, composition and/or activity of (V) in a host ***organism*** .

EXAMPLE - Lactobacillus rhamnosus strain HN001 DNA libraries were constructed and screened as follows. DNA was prepared in large scale by cultivating the bacteria in MRS broth (undefined) and 1 ml Lactobacillus glycerol stock as inoculum, and incubating at 37 degrees C for approximately 16 hours with shaking. The cultures were centrifuged to pellet the cells. The cell pellet was resuspended in 40 ml fresh MRS broth. Fresh MRS broth was added and the culture was incubated for a further 2 hours at 37 degrees C with shaking. The cells were pelleted by centrifugation and supernatant removed. Cell pellets were washed twice in 20 ml buffer A (50 mM NaCl, 30 mM Tris pH 8.0,0.5 mM ethylenediamine tetraacetic-acid (EDTA)). Cells were resuspended in 2.5 ml buffer B (25% sucrose (w/v), 50 mM Tris pH 8.0,1 mM EDTA, 20 mg/ml lysozyme, 20 microg/ml mutanolysin) and incubated at 37 degrees C for 45 min. Equal volumes of EDTA was added to each tube and allowed to incubate at room temperature for 5 minutes. 30% sodium dodecylsulfate (SDS) solution was added, mixed and incubated at 65 degrees C. 50 microl Proteinase K from a stock solution of 20 mg/ml was added and tubes incubated at 65 degrees C for 15 minutes. DNA was extracted, centrifuged and the aqueous phase was removed. Crude DNA was precipitated. After resuspension in 500 microl TE buffer, DNase-free RNase was added to a final concentration of 100 microg/ml and incubated at 37 degrees C for 30 minutes (min). The incubation was extended for a further 30 min after adding 100 microl ProteinaseK. DNA was digested with Sau3AI. Following incubation for 1 hour at 37 degrees C, DNA was divided into two tubes. 31 microl 0.5 M EDTA was added to stop the digestion and 17 microl samples were taken for agarose gel analysis. Sucrose gradient size fractionation was conducted as follows. 100ml of 50% sucrose (w/v) was made in TEN buffer (1M NaCl, 20 mM Tris pH 8.0, 5 mM EDTA) and sterile filtered. Dilutions of sucrose were prepared and overlaid and kept overnight at 4 degrees C. TEN buffer (4 ml) was loaded onto the gradient, with 3 ml of DNA solution on top. The gradients were centrifuged at 26K for 18 hours at 4 degrees C. Agarose gel was used to analyze the fractions. The best two pairs of fractions were pooled and diluted to contain less than 10% sucrose. TEN buffer was added and DNA precipitated and an overnight incubation at -20 degrees C. DNA pellets were resuspended in 300 microl TE buffer and re-precipitated. DNA was pelleted washed with 70% ethanol and pelleted again, dried and resuspended in 10 microl TE buffer. DNA was ligated into dephosphorylated BamHI-digested pBluescript SK II+ and dephosphorylated BamHI-digested lambda ZAP ***Express*** . Mass excision from the primary packaged phage library was done using XL1-Blue MRF cells and EXAssist Helper. The excised phagemids were diluted with NZY broth and plated out onto LB-kanamycin agar plates containing 5-bromo-4-chloro-3-indolyl-beta-D-galactoside (X-gal) and isopropylthio-beta-galactoside (IPTG). After incubation, single colonies were picked for polymerase chain reaction (PCR) size determination. Of the colonies picked for DNA minipreps and subsequent sequencing, the large majority contained an insert suitable for sequencing. Positive colonies were cultured in Luria Bertani (LB) broth with kanamycin or ampicillin depending on the vector

used, and DNA was purified by means of rapid alkaline lysis minipreps. Agarose gels at 1% were used to screen sequencing templates for chromosomal contamination and concentration. Dye terminator sequencing reactions were prepared. To extend the sequences of the inserts from these clones, primers were designed from the determined nucleotide sequences so that the primer sequences were located approximately 100 base pairs (bp) downstream of 5' end and 100 bp upstream of the 3' end of the determined nucleotide ***sequence*** . Selection of primers were done with the Gap4 Genome Assembly Program. The determined nucleotide sequences have fully defined ***sequence*** of any one of 59 (400-2100) bp sequences all given in the specification, and encode an amino acid ***sequence*** of any one of 59 (150-700) amino acid sequences all given in the specifications. (128 pages)

L13 ANSWER 7 OF 10 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:114714 BIOSIS
DOCUMENT NUMBER: PREV200100114714
TITLE: A novel promoter architecture for microaerobic activation by the anaerobic transcription factor FNR.
AUTHOR(S): Marshall, Fiona A.; Messenger, Sarah L.; Wyborn, Neil R.; Guest, John R.; Wing, Helen; Busby, Stephen J. W.; Green, Jeffrey [Reprint author]
CORPORATE SOURCE: Department of Molecular Biology and Biotechnology, Krebs Institute for Biomolecular Research, University of Sheffield, Sheffield, S10 2TN, UK
jeff.green@sheffield.ac.uk
SOURCE: Molecular Microbiology, (February, 2001) Vol. 39, No. 3, pp. 747-753. print.
CODEN: MOMIEE. ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Mar 2001
Last Updated on STN: 15 Feb 2002
AB The yfiD gene of Escherichia coli has an unusual promoter architecture in which an FNR dimer located at -93.5 inhibits transcription activation mediated by another FNR dimer bound at the typical class II position (-40.5). In vitro transcription from the yfiD promoter indicated that FNR alone can downregulate yfiD expression. Analysis of yfiD::lac reporters showed that five turns of the DNA helix between FNR sites was optimal for downregulation. FNR heterodimers, in which one subunit carried a defective repression surface, revealed that the upstream subunit of the -40.5 dimer and the downstream subunit of the -93.5 dimer were most important for downregulating yfiD expression. Deletion of the C-terminal domain of the alpha-subunit of RNA polymerase (RNAP) did not affect FNR-mediated repression, suggesting that repression is mediated through FNR-FNR and not FNR-RNAP interactions. Maximum yfiD::lac expression was observed in cultures exposed to 10 muM oxygen. More or less oxygen reduced expression dramatically. This pattern of response was dependent on the combination of a high-affinity site at the activating class II position and a lower affinity site at the upstream position.

L13 ANSWER 8 OF 10 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-10149 BIOTECHDS
TITLE: New method for producing carboxylic acids; carboxylic acid, e.g. malic acid preparation by malic enzyme expression in Escherichia coli or Lactobacillus sp.
AUTHOR: Nghiem N P; Donnelly M; Millard C S; Stols L
PATENT ASSIGNEE: Lockheed-Martin-Energy-Systems; Univ.Chicago
LOCATION: Oak Ridge, TN, USA; Chicago, IL, USA.
PATENT INFO: WO 9833930 6 Aug 1998
APPLICATION INFO: WO 1998-US1877 30 Jan 1998
PRIORITY INFO: US 1997-792655 31 Jan 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-437481 [37]
AN 1998-10149 BIOTECHDS
AB A new method for producing carboxylic acids (e.g. malic acid) involves: inoculating a medium containing a C-source with a carboxylic acid-producing ***microorganism*** ; incubating the ***microorganism*** in an aerobic atmosphere to promote rapid growth of

the ***microorganism*** , thereby increasing its biomass; controllably releasing oxygen to maintain the aerobic atmosphere; controllably feeding the ***microorganism*** a solution containing the C-source to maintain a concentration of the C-source within the medium of about 0.5 to 1 g/l; depriving the aerobic atmosphere of oxygen to produce an anaerobic atmosphere to cause the ***microorganism*** to undergo anaerobic metabolism; controllably feeding the ***microorganism*** the solution containing the C-source to maintain a concentration of the C-source within the medium of at least 1 g/l; and converting the C-source to carboxylic acids using the anaerobic metabolism of the ***microorganism*** . The ***microorganism*** is preferably Escherichia coli AFP-111 lacking the ***gene*** for ***formate*** - ***C*** - ***acetyltransferase*** (EC-2.3.1.54) and lactate-dehydrogenase (EC-1.1.1.27), or Lactobacillus sp., both of which are genetically engineered to ***express*** a malic enzyme. (34pp)

L13 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1991:222839 CAPLUS
DOCUMENT NUMBER: 114:222839
TITLE: A strong regulatable promoter for expression of heterologous genes in facultatively anaerobic bacteria
INVENTOR(S): Boeck, August; Sawers, Robert Gary; Jarsch, Michael; Herbst, Roland
PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany
SOURCE: Eur. Pat. Appl., 28 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 393684	A1	19901024	EP 1990-107465	19900419
EP 393684	B1	19970618		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
DE 3926076	A1	19901025	DE 1989-3926076	19890807
AT 154639	E	19970715	AT 1990-107465	19900419
ES 2104568	T3	19971016	ES 1990-107465	19900419
CA 2015046	AA	19901021	CA 1990-2015046	19900420
CA 2015046	C	20030916		
JP 03080088	A2	19910404	JP 1990-103227	19900420
US 5830720	A	19981103	US 1995-386198	19950209

PRIORITY APPLN. INFO.: DE 1989-3913201 A 19890421
DE 1989-3926076 A 19890807
US 1990-503593 B2 19900403
US 1993-8311 B1 19930125

AB The promoter of a ***pyruvate*** - ***formate*** ***lyase*** ***gene*** (source ***organism*** not given) that shows strong induction by pyruvate and repression by oxygen that is suitable for regulation of ***expression*** of heterologous genes in fermn. by facultatively anaerobic bacteria that carry a functional fnr ***gene*** is described. Analogs of the promoter with deletions are described that show stronger induction than the wild-type promoter. A series of constructs in which promoters with deletions in the promoter were constructed and levels of induction and repression tested using lacZ as a reporter gene. Under repressing conditions (aerobic) the deleted promoters showed higher basal levels of expression than the wild type promoter (e.g. .apprx.9,000 units vs. 200-400) and higher induced levels of expression (>45,000 units vs. .ltoreq.6,700). Induction ratios were not necessarily greater but all inductions required a functional fnr gene. Use of one of these promoters to drive expression of a creatinase gene in a fermn. vessel showed a strong induction of creatinase synthesis as the dissolved O fell.

L13 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1989:19125 CAPLUS
DOCUMENT NUMBER: 110:19125
TITLE: Anaerobic regulation of pyruvate formate-lyase from Escherichia coli K-12

AUTHOR(S): Sawers, Gary; Boeck, August
CORPORATE SOURCE: Univ. Muenchen, Munich, D-8000, Fed. Rep. Ger.
SOURCE: Journal of Bacteriology (1988), 170(11), 5330-6
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The anaerobic regulation of the gene encoding pyruvate formate-lyase from E. coli was investigated. Expression of a pfl'-lacZ protein fusion demonstrated that the gene is subject to a 12-fold anaerobic induction which can be stimulated a further 2-fold by the addn. of pyruvate to the growth medium. Construction of a strain deleted for pfl verified that either pyruvate or a metabolite of glycolysis functions as an inducer of pfl gene expression. Complete anaerobic induction required the presence of a functional fnr gene product. However, the dependence was not abs. since a 2-3-fold anaerobic induction could still be obsd. in an fnr mutant. These results could be confirmed immunol. by analyzing the levels of pyruvate formate-lyase protein present in cells grown under various conditions. It was also shown that pfl'-'lacZ expression was partially repressed by nitrate and that this repression was mediated by the narL gene product.

=> d ibib abs L14 1-3

L14 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-15593 BIOTECHDS
TITLE: Producing D-lactic acid, involves culturing bacteria having inactivated or lowered pyruvate formate-lyase activity in culture medium containing two or more amino acids and recovering lactic acid from culture;
lactic acid production via bacterium culture medium
AUTHOR: WADA M; OIKAWA T; MOCHIZUKI D; TOKUDA J; KAWASHIMA M; ARAKI T; ABE R; MIYAKE H; TAKAHASHI H; SAWAI H; MIMIZUKA T; MORISHIGE T; HIGASHI Y
PATENT ASSIGNEE: MITSUI CHEM INC
PATENT INFO: WO 2005033324 14 Apr 2005
APPLICATION INFO: WO 2004-JP14037 17 Sep 2004
PRIORITY INFO: JP 2004-150253 20 May 2004; JP 2003-340062 30 Sep 2003
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2005-315387 [32]
AN 2005-15593 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) lactic acid, involves culturing a heterolactic fermentation bacteria having inactivated or lowered ***pyruvate*** ***formate*** - ***lyase*** (pfl) activity in a culture medium containing two or more amino acids and recovering lactic acid from the culture.

DETAILED DESCRIPTION - Producing (M1): (a) lactic acid, involves culturing a heterolactic fermentation bacteria having inactivated or lowered ***pyruvate*** ***formate*** - ***lyase*** (pfl) activity in a culture medium containing two or more amino acids and recovering lactic acid from the culture; or (b) D-lactic acid, involves: (i) culturing a bacteria having inactivated or lowered pfl activity and elevated Escherichia coli-origin NADH-dependent D-lactic acid dehydrogenase (ldhA) activity and recovering D-lactic acid from the culture; or (ii) culturing ***microorganism*** having inactivated or lowered FAD-dependent D-lactic acid dehydrogenase (dld) activity in a liquid medium, producing and accumulating D-lactic acid in the culture solution and isolating D-lactic acid from the culture solution.
INDEPENDENT CLAIMS are also included for: (1) microorganisms (I) having inactivated or lowered dld activity, pfl activity and/or enhanced ldhA activity; (2) microorganisms (II) having enhanced ldhA activity, where the activity is enhanced by ligating a ***gene*** encoding ldhA to a promoter of a ***gene*** controlling the ***expression*** of a protein, which participate in a glycolysis system, a nucleic acid ***biosynthesis*** system or an ***amino*** ***acid*** ***biosynthesis*** system, on genome; and (3) microorganisms (III) having inactivated or lowered pfl and dld activity, and having a tricarboxylic acid (TCA) cycle and inactivated or lowered malate

dehydrogenase (mdh) activity.

BIOTECHNOLOGY - Preferred Method: In (M1)-(a), the bacteria is E.coli MT-10934 (FERM BP-10057) strain. In (M1)-(b)-(i), the bacteria is E.coli and the culture medium contains two or more amino acids. In (M1), the culture is carried out on the following conditions, aerobic condition, 30degreesC temperature, pH ranging from 6-8 and oxygen volumetric coefficient (KLa) of 1 h-1 or more and 400 h-1 or less at normal pressure. Preferred ***Microorganism*** : (II) has inactivated or lowered dld and pfl activity. (II) is E.coli and the promoter is the promoter of glyceraldehyde 3 phosphate dehydrogenase ***gene*** derived from E.coli. (III) has inactivated or lowered aspartic acid ammonia-lyase (aspA) activity. (III) is E.coli and has enhanced ldhA activity.

USE - (M1) or (I)-(III) is useful for producing D-lactic acid. (III) is useful for producing compound (preferably D-lactic acid) other than organic acid produced in the TCA cycle, which involves culturing (III) in a culture medium and recovering the product (claimed).

ADVANTAGE - (M1) is a highly selected process that enables efficient production of D-lactic acid having a high optical purity with the formation of little organic acids as by products. (75 pages)

L14 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN DUPLICATE 1

ACCESSION NUMBER: 2004-25480 BIOTECHDS

TITLE: Production of L-amino acids e.g. L-threonine by fermentation involves culturing recombinant family Enterobacteriaceae microorganisms producing L-amino acid and having overexpressed yfiD open reading frame and/or pfl B gene; and isolating;
amino acid production via plasmid expression in host cell culture

AUTHOR: RIEPING M; FARWICK M

PATENT ASSIGNEE: DEGUSSA AG

PATENT INFO: WO 2004090149 21 Oct 2004

APPLICATION INFO: WO 2004-EP3207 26 Mar 2004

PRIORITY INFO: DE 2003-1016109 9 Apr 2003; DE 2003-1016109 9 Apr 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-757988 [74]

AN 2004-25480 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Production of L-amino acids by fermentation of recombinant ***Enterobacteriaceae*** involves culturing microorganisms producing ***L*** - ***amino*** ***acid*** and having overexpressed ***yfiD*** open reading frame (ORF) and/or pfl B ***gene*** or nucleotide sequences coding for the ***gene*** products; and isolating ***amino*** ***acid*** .

DETAILED DESCRIPTION - Production of L-amino acids by fermentation of recombinant microorganisms of ***Enterobacteriaceae*** family involves: (1) culturing microorganisms producing ***L*** - ***amino*** ***acid*** and having overexpressed ***yfiD*** open reading frame (ORF) and/or pfl B ***gene*** or nucleotide sequences coding for the ***gene*** products; and (2) isolating ***amino*** ***acid*** in which optional constituents of the fermentation broth and/or entire or portions (0 - 100%) of the biomass, optionally remain. INDEPENDENT CLAIMS are included for the following: (1) method (M) for the production of L-threonine involving fermenting microorganisms of ***Enterobacteriaceae*** family having enhanced genes for the biosynthetic pathway of L-threonine selected from at least one of: (a) the thrABC operon coding for aspartate kinase, homoserine dehydrogenase, homoserine kinase or threonine synthase; the pyc ***gene*** coding for pyruvate carboxylase; (b) the pps ***gene*** for phosphoenolpyruvate synthase; (c) the ppc ***gene*** coding for phosphoenolpyruvate carboxylase; (d) the genes pntA ad pntB coding for transhydrogenases; (e) the ***gene*** rhtB imparting homoserine resistance; (f) the mqo ***gene*** coding for malate:quinone oxidoreductase; (g) the ***gene*** rhtC imparting threonine resistance; (h) the thrE ***gene*** coding for the threonine export protein; (i) the gdhA ***gene*** coding for glutamate dehydrogenase; (j) the hns ***gene*** coding for the DNA bonding protein HLP-II; (k)

the pgm ***gene*** phosphoglucomutase; (l) the fba ***gene*** coding for fructose biphosphate aldolase; (m) the ptsH ***gene*** coding for phosphohistidine protein hexose phosphotranaferase; (n) the ptsI ***gene*** coding for enzyme I of the phosphotransferase system; (o) the crr ***gene*** coding for the glucose-specific IIA component; (p) the ptsG ***gene*** coding for the glucose-specific IIBC component; (q) the irp ***gene*** coding for the regulator of the leucine regulon; (r) the csrA ***gene*** coding for the global regulator Csr; (s) the fadA ***gene*** coding for the regulator of the fad regulon; (t) the iclR ***gene*** coding for the regulator of central intermediary metabolism; (u) the mopB ***gene*** coding for the 10 kDa chaperon; (v) the ahpC ***gene*** coding for the small subunit of alkyl hydroperoxide reductase; (w) the ahpF ***gene*** coding for the large subunit of alkyl hydroperoxide reductase; (x) the cysK ***gene*** coding for cysteine synthase A; (y) the cysB ***gene*** coding for the regulator of the cys regulon; (z) the cysJ ***gene*** coding for the flavoprotein of NADPH sulfite reductase; (aa) the cysH ***gene*** coding for adenylyl sulfate reductase; (bb) the phoR ***gene*** coding for the positive regulator PhoB of the pho regulon; (cc) the phoR ***gene*** coding for the sensor protein of the pho regulon; (dd) the phoE ***gene*** coding for the protein E of the outer cell membrane; (ee) the pykF ***gene*** coding for pyruvate kinase I, which is stimulated by fructose; (ff) the pfkB ***gene*** coding for 6-phosphofructokinase II; (gg) the male ***gene*** coding for the periplasmic binding protein of maltose transport; (hh) the soda ***gene*** coding for superoxide dismutase; (ii) the rseA ***gene*** coding for a membrane protein with anti-sigmaE activity; (jj) the rseC ***gene*** coding for a global regulator of the sigmaE factor; (kk) the sucA ***gene*** coding for the decarboxylase subunit of 2-ketoglutarate dehydrogenase; (ll) the sucB ***gene*** coding for the dihydrolipoyl transsuccinase E2 subunit of 2-ketoglutarate dehydrogenase; (mm) the sucC ***gene*** coding for the beta-subunit of succinyl-CoA synthetase; (nn) the sucD ***gene*** coding for the alpha-subunit of succinyl-CoA synthetase; (oo) the adk ***gene*** coding for adenylate kinase; (pp) the hdeA ***gene*** coding for a periplasmic protein with chaperonin-type function; (qq) the icd ***gene*** coding for isocitrate dehydrogenase; (rr) the mglB ***gene*** coding for the periplasmic galactose-binding transport protein; (ss) the lpd ***gene*** coding for dihydrolipoamide dehydrogenase; (tt) the aceE ***gene*** coding for the E1 component of the pyruvate-dehydrogenase complex; (uu) the sceF ***gene*** coding for the E2 component of the pyruvate-dehydrogenase complex; (vv) the pepB ***gene*** coding for aminopeptidase B; (ww) the aldH ***gene*** coding for aldehyde dehydrogenase; (xx) the bfr ***gene*** coding for the iron-storage homoprotein; (yy) the udp ***gene*** coding for uridine phosphorylase; or (zz) the resB ***gene*** coding for the regulator of sigmaE-factor activity; and (2) microorganisms of the ***Enterobacteriaceae*** family (preferably genus Escherichia)) in which the ***yfiD*** ORF and/or the pflB ***gene*** or nucleotide ***sequence*** coding for their ***gene*** product are present in enhanced or overexpressed form.

BIOTECHNOLOGY - Preferred Microorganisms: The recombinant microorganisms are generated by the transformation of a ***microorganism*** of the ***Enterobacteriaceae*** family with a vector containing ***yfiD*** ORF and/or plfB ***gene*** ; so that the number of copies of the plfB ***gene*** (s) and/or ***yfiD*** ORF is/are increased by at least 1. The ***yfiD*** ORF and/or pflB ***gene*** used is under the control of a promoter. Through the enhancement of the ***yfiD*** ORF and/or pflB ***gene*** , the concentration of activity of the ***yfiD*** ***gene*** product and/or the pfiB ***gene*** product (protein) is increased by at least 10%, relative to the activity or concentration of the ***gene*** product in the initial strain. The ***microorganism*** is selected from Escherichia, Erwinia, Providencia or Serratia. The microorganisms further have overexpressed genes of the biosynthetic pathway of the desired ***L*** - ***amino*** ***acid*** , and have the metabolic pathways that diminish the formation of the desired ***L*** - ***amino*** ***acid*** , at least partially eliminated. In method (M). The microorganisms further have, simultaneously, ***gene*** selected from at least one of the ***gene*** coding for threonine

dehydrogenase; the mdh ***gene*** coding for malate dehydrogenase, the ***gene*** product of the yifA ORF; the ***gene*** product of ytfP ORF; the pckA ***gene*** coding for phosphoenolpyruvate carboxykinase; the poxB ***gene*** coding for pyruvate oxidase; the aceA ***gene*** coding for isocitrate lyase; the dgsA ***gene*** coding for the DgsA regulator of the phosphotransferase system; the fruR ***gene*** coding for fructose repressor; the rpoS ***gene*** coding for the sigma38 factor; the aspA ***gene*** coding for aspartate ammonium lyase; or the aceB ***gene*** coding for malate synthase A, is/are attenuated, eliminated, or their ***expression*** is diminished. Preferred Method: The increase in the number of copies of the ***yfiD*** ORF and/or the pflB ***gene*** by at least 1 is achieved by extrachromosomal replication of the vector and involves: mutation of the promoter and regulation regions or the ribosome binding site upstream of the ***yfiD*** ORF and/or pflB ***gene*** ; or incorporating ***expression*** cassettes or promoters upstream of the ***yfiD*** ORF and/or pflB ***gene*** .

USE - For the production of L-amino acids (such as L-asparagine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine) by fermentation of recombinant microorganisms (claimed).

ADVANTAGE - The method results in an enhanced production of L-amino acids. The enhancement of ***yfiD*** ORF and pflB ***gene*** enhances the enzyme(s) of the known threonine- ***biosynthesis*** pathway, enzymes of the anaplerotic metabolism, enzymes for the production of reduced nicotinamide adenine dinucleotide phosphate, enzymes of glycolysis, PTS enzymes and/or enzymes of sulfur metabolism, and hence the production of L-amino acids.

EXAMPLE - Production of L-threonine with the strain MG422/pTrc99AyfiD was determined. The L-threonine producing E. coli strain MG442 as described in US278 was selected. The strain was transformed with an ***expression*** plasmid pTrc99AyfiD and with a vector pTrc99A and the plasmid bearing cells were selected on LB agar with ampicillin (50 microg/ml), to obtain strains MG443/pTrc99Ayfid and MG442/pTrc99A. The selected single colonies were subsequently multiplied further on minimal medium. The formation of threonine was examined in batch cultures (10 ml). At the end, a preculture medium (10 ml) was inoculated and incubated for 16 hours at 37 degrees C at shaker. At the time of preculture, the medium (250 micro l) was incubated into a production medium (10 ml) and incubated for 48 hours at 37 degrees C and the L-threonine formed was measured. Comparatively, the formation of L-threonine by initial strain MG442 was examined without the addition ampicillin. The L-threonine produced (g/l) by the strains MG442/pTrc99Ayfid/MG/pTrc99A/MG442 was found to be 2.5/1.3/1.4. (52 pages)

L14 ANSWER 3 OF 3 USPATFULL on STN
ACCESSION NUMBER: 2004:299255 USPATFULL
TITLE: Process for the production of L-amino acids using strains of the enterobacteriaceae family
INVENTOR(S): Rieping, Mechthild, Bielefeld, GERMANY, FEDERAL REPUBLIC OF
Farwick, Mike, Essen, GERMANY, FEDERAL REPUBLIC OF

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004235122	A1	20041125
APPLICATION INFO.:	US 2004-817431	A1	20040405 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	DE 2003-10316109	20030409

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FITCH, EVEN, TABIN & FLANNERY, SUITE 401L, 1801 K STREET, NW, WASHINGTON, DC, 20006-1201
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Page(s)

LINE COUNT: 1402
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a process for the production of L-amino acids by fermentation of recombinant microorganisms of the Enterobacteriaceae family, wherein

a) the yfiD ORF and/or the pflB gene or nucleotide sequences coding for the gene products are overexpressed in the microorganisms producing the desired L-amino acid, and the microorganisms are cultured in a medium under conditions in which the desired L-amino acid is enriched in the medium or in the cells; and

b) the desired L-amino acid is isolated, in a manner such that constituents of the fermentation broth and/or the biomass in its entirety or in portions (>0 to 100%) either remain in the isolated product or are completely removed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

L1 QUE ((FORMATE(W) C-ACETYLTRANSFERASE#) OR (FORMATE(W) ACETYLTRA

FILE 'SCISEARCH, CAPLUS, BIOSIS, MEDLINE, EMBASE, LIFESCI, USPATFULL, ESBIOBASE, BIOTECHNO, PASCAL, TOXCENTER, BIOTECHDS, BIOENG, AGRICOLA, WPIDS' ENTERED AT 12:17:21 ON 11 APR 2006

L2 2487 S L1
L3 624 S (GENE OR SEQUENCE OR POLYNUCLEOTIDE OR CLONE)(S)L2
L4 250 S EXPRESS?(S)L3
L7 9 S ((L-AMINO (W) ACID) OR (AMINO (W) ACID)(S) BIOSYNTHESIS)(S)L2
L9 12 S (MICROORGANISM OR ORGANISM OR ENTEROBACTERIACEAE)(S)L4
L10 4 S (MICROORGANISM OR ORGANISM OR ENTEROBACTERIACEAE)(S)L7
L11 1 S (THRABC(W)OPERON)(S)L4
L12 5 DUP REM L7 (4 DUPLICATES REMOVED)
L13 10 DUP REM L9 (2 DUPLICATES REMOVED)
L14 3 DUP REM L10 (1 DUPLICATE REMOVED)

=> log y